Methods

The objective of Task 5, WA 4-11 was to evaluate the intra- and inter-laboratory variability resulting from five independent laboratories conducting three separate saturation and competitive binding assays using individual laboratory prepared "standard" cytosol. The mean and coefficient of variation (CV) within and between laboratory results in measurement of K_d, number of receptors, and B_{max} from a saturation assay was evaluated to ensure that each laboratory was preparing the rat uterine cytosol correctly and could reliably measure the relevant descriptors. In addition, the variability in the competitive binding assay was calculated from the measurements of IC_{50} for R1881 and the weak binder (dexamethasone) and the relative binding affinity (RBA) for the weak binder. The goodness-of-fit (R^2 values ranging from 0 to 1) to the appropriate nonlinear binding equations were calculated. Finally, the sources of variability for the observed differences in laboratory results were examined.

Intra-laboratory variability of the resulting measurements was defined as the CV (standard deviation/mean x 100%) between the three separate assays (indicated by the date of the run). Inter-laboratory variability was defined as the CV between the mean laboratory statistics (average of the three runs). Other sources of variability associated with the estimation process of these statistics include the non-specific binding goodness-of-fit to a simple linear model for both the saturation and competitive binding assays and the variability in the activity of the radioactive labels.

Observations were removed from data analysis by the submitting laboratory based on their determination of outliers and level of saturation. Observations were removed from the intra- and inter laboratory comparison to allow convergence of the nonlinear one-site binding or competitive binding equations. The criteria used for model convergence and an appropriate measurement of the assay parameters were an R^2 value between 0 and 1, a $K_d > 0$, for the saturation assay, and a within replicate CV of less than 30%. Outliers that were not removed by the submitting laboratory and did not affect model convergence were indicated, and the statistical analysis was conducted with and without them.

Results

Saturation binding Assay: Each of the five participating laboratories conducted three independent runs of the saturation assay with triplicate tubes of each concentration. The data used for the intra- and inter-laboratory comparison are presented in Appendix A. Two of the runs from Lab D did not converge and one run had a poor goodness-of-fit ($R^2 = 0.46$) and they were not used in the statistical analysis. The data from these runs, however, were included in the plots. The goodness-of-fit to the one-site binding equation ranged from 0.86 to 1.00 with a median value of 0.95 for the remaining 12 runs (Table 1). The range of B_{max} (fmole/100 μg) values was 4.81 to 16.8 with a median value of 9.25. The range of K_d (nM) values was 0.660 to 2.88 with a median value of 0.928. The intra-laboratory CVs for B_{max} ranged from 4.4% to 11% with a median of 6.9% and for K_d ranged from 2.9% to 24% with a median of 8.3%.

Table 1. Intra-Laboratory variability of the statistics associated with the saturation assay. The results from Lab D were not used in the statistical calculations.

Statistic	Assay	Lab A	Lab B	Lab C	Lab D	Lab E
Bmax (fmole/100 μg)	1	6.24	5.30	14.3		14.4
Bmax (fmole/100 μg)	2	6.10	4.81	16.8		12.3
Bmax (fmole/100 μg)	3	5.72	5.32	15.3	0.611	15.6
Kd (nM)	1	1.92	0.699	0.835		0.930
Kd (nM)	2	2.00	0.677	0.930		0.834
Kd (nM)	3	2.88	0.660	1.03	0.307	0.926
Goodness of Fit	1	0.95	0.94	0.98	Did not Converge	0.97
Goodness of Fit	2	0.96	0.92	0.97	Did not Converge	0.86
Goodness of Fit	3	0.95	0.88	0.98	0.46	1.00
		Lab A	**Lab B**	**Lab C**	**Lab D**	**Lab E**
Mean Bmax (fmole/100 μg)		6.02	5.14	15.4	0.61	14.1
CV Bmax		4.4%	5.6%	8.1%	NA	11.9%
Mean Kd (nM)		2.27	0.68	0.93	0.31	0.90
CV Kd		23.5%	2.9%	10.4%	NA	6.1%
Average Goodness of Fit		0.95	0.91	0.98	0.46	0.94

The inter-laboratory variability of the saturation binding measurements from the four laboratories was 53% and 61% for B_{max} and K_d, respectively (Table 2). The variability in these measurements was large and can be explained by the variability in the fitted one-site binding curves which resulted from each laboratories interpretation and reproduction of the saturation assay protocol (Figure 1).

Table 2. Inter-Laboratory variability of the statistics associated with the saturation assay. The results from Lab D were not used in the statistical calculations.

Statistic	Bmax (fmole/100 μg)	Kd
Mean	10.2	1.19
CV	53%	61%

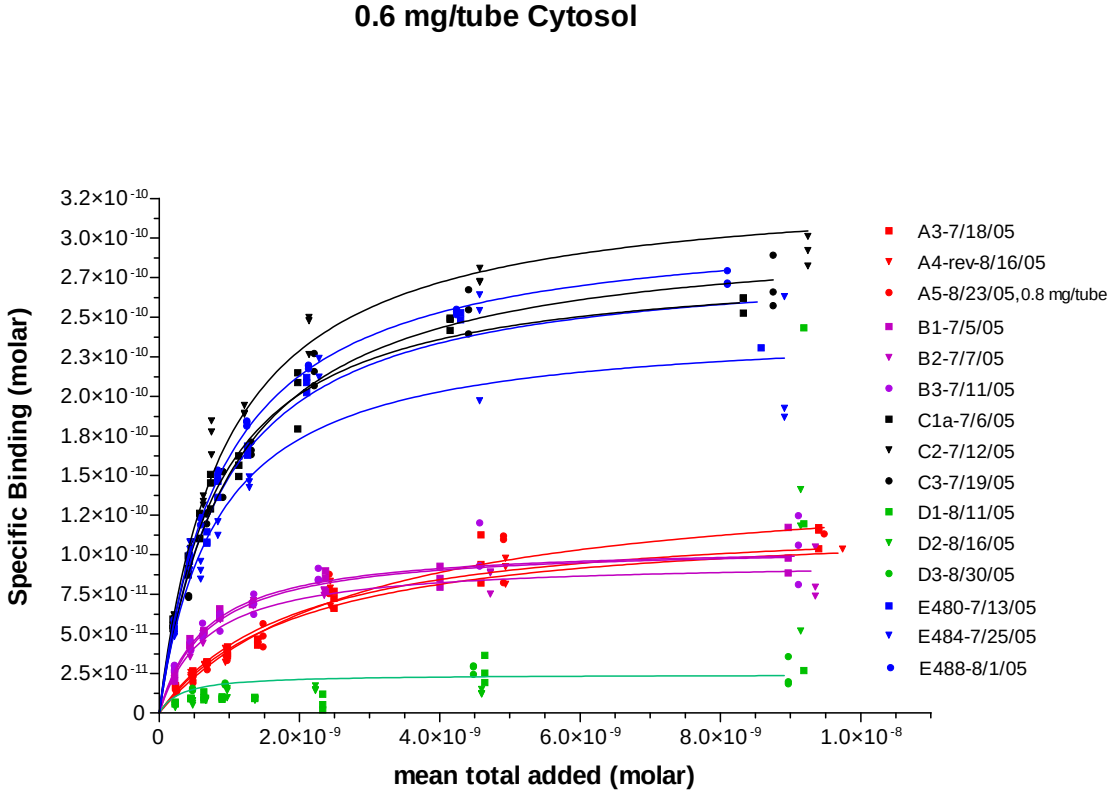

Figure 1. Inter-laboratory variability of one-site binding curves. The fitted curve for run C2-2/24/05 is without the designated outlier.

Competitive Binding Assay: Only three of the participating laboratories conducted three independent runs of the competitive binding assay with a standard and a weak positive control with triplicate tubes of each concentration. The data used for the intra- and inter-laboratory comparison are presented in Appendix B. One data point from Lab C Run 6-9/1/05 for the standard was considered an outlier and the statistical analysis was conducted with and without this observation.

The goodness-of-fit to the one-site competition equation for the standard ranged from 0.65 to 1.00 with a median value of .99 for the 9 runs (Table 3). Without the outlier the goodness-of-fit for Lab C Run 6-9/1/05 increases from 0.65 to 0.81. However, the percentage R1881 bound for this run was suppressed at the lower competitor concentrations (Figure 2). If the top of the curve is not constrained to 100% bound, then the goodness of fit becomes 0.99. The goodness-of-fit for the weak positive control ranged from 0.11 to 1.00 with a median value of 0.99. The low goodness-of-fit was associated with the percent bound being suppressed for Lab C Run 6-9/1/05 (Figure 3). Again, if the top of the curve is not constrained to 100% bound, then the goodness of fit becomes 1.00.

Table 3. Intra-Laboratory variability of the statistics associated with the competitive assay with and without an identified outlier and modeling constraints shown in parentheses

Statistic	Rep	**Lab B**	**Lab C**	**Lab E**
IC50 Standard	1	1.32E-09	8.86E-10	1.48E-09
IC50 Standard	2	1.36E-09	1.95E-09	1.55E-09
IC50 Standard	3	1.76E-09	6.95E-10 (1.34E-09)	1.64E-09
IC50 Weak Positive	1	5.73E-05	5.77E-05	4.89E-05
IC50 Weak Positive	2	5.83E-05	7.69E-05	5.04E-05
IC50 Weak Positive	3	7.47E-05	6.98E-05 (9.04E-05)	4.92E-05
RBA	1	0.002%	0.002%	0.003%
RBA	2	0.002%	0.003%	0.003%
RBA	3	0.002%	0.001% (0.002%)	0.003%
Goodness of Fit Standard	1	0.99	0.98	1.00
Goodness of Fit Standard	2	1.00	0.99	1.00
Goodness of Fit Standard	3	0.99	0.65 (0.99)	1.00
Goodness of Fit Weak Positive	1	0.99	0.99	0.98
Goodness of Fit Weak Positive	2	0.98	0.99	1.00
Goodness of Fit Weak Positive	3	0.97	0.11 (0.94)	0.99
		Lab B	**Lab C**	**Lab E**
Mean IC50 Standard		1.48E-09	1.18E-09 (1.39E-09)	1.55E-09
CV IC50 Standard		17%	57% (38%)	5%
Mean IC50 Weak Positive		6.34E-05	6.82E-05 (7.50E-05)	4.95E-05
CV log IC50 Weak Positive		15%	14% (22%)	2%
Average RBA		0.002%	0.002% (0.002%)	0.003%
CV RBA		1%	46% (32%)	5%
Average Goodness of Fit Standard		99%	87% (99%)	100%
Average Goodness of Fit Weak Positive		98%	70% (97%)	99%

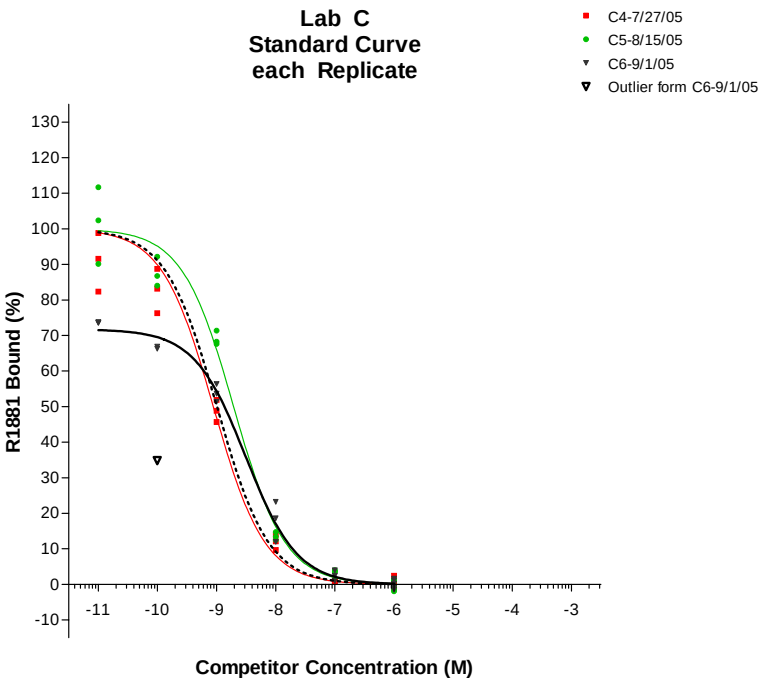

Figure 2. Lab C one-site competitive curves for the standard R1881 with and without the top parameter constrained to 100% (dashed and solid black lines respectively)

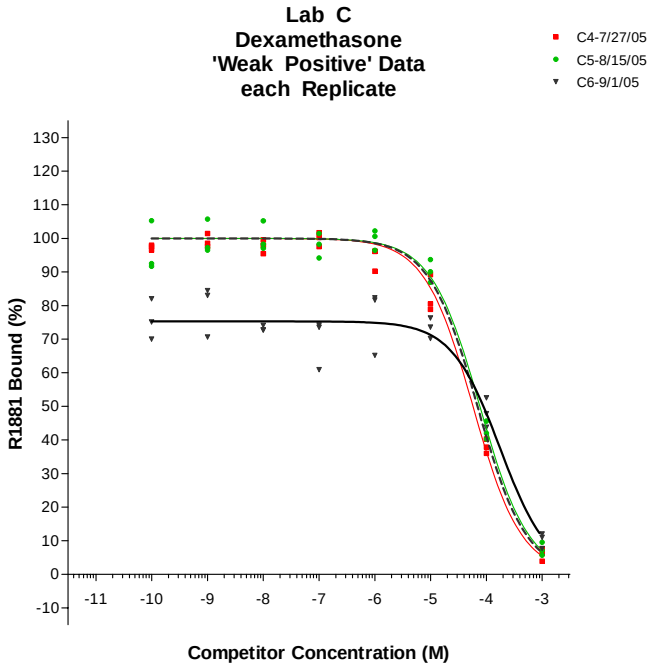

Figure 3. Lab C one-site competitive curves for the weak positive with and without the top parameter constrained to 100% (dashed and solid black lines respectively)

With all of the data and normal model constraints, the range of IC_{50} values for the standard was 6.95E-10 to 1.95E-09 with a median value of 1.48E-09. The range of IC_{50} values for the weak positive control was 4.89E-05 to 7.69E-05 with a median value of 5.77E-05. The resulting RBAs ranged from 0.0010% to 0.0033% with a median value of 0.0024%. The intra-laboratory CVs for RBA ranged from 1% to 46% with a median of 5%.

When the identified data point was removed from the analysis and the 100% bound constraint on the model was removed from both the standard and weak positive curve fitting for Lab C Run 6-9/1/05, the resulting IC_{50} values were maintained at 50% bound even though they were associated with a higher point on the curve (at approximately 70% of the estimated top of the curve). The CVs for the IC50 values for the standard and the weak positive decreased 19% and increased 8% respectively (Table 3). The CV for RBA decreased 14%.

With all of the data and normal model constraints, the inter-laboratory variability of the three competitive binding measurements was 14%, 16%, and 30% for the standard and weak positive log IC_{50} values and RBA, respectively (Table 4). When the identified data point was removed from the analysis and the 100% bound constraint on the model was removed from both the standard and weak positive curve fitting for Lab C Run 6-9/1/05, the CV dropped only slightly for the RBA. The variability in these measurements was can be inferred by the variability in the fitted one-site competitive curves (Figure 4).

Table 4. Inter-Laboratory variability of the statistics associated with the competitive binding assay with and without an identified outlier and modeling constraints shown in parentheses

Statistic	IC50 Standard	IC50 Weak Positive	RBA
Mean	1.40E-09 (1.47E-09)	6.04E-05 (6.27E-05)	0.002% (0.002%)
CV	14% (5%)	16% (20%)	30% (27%)

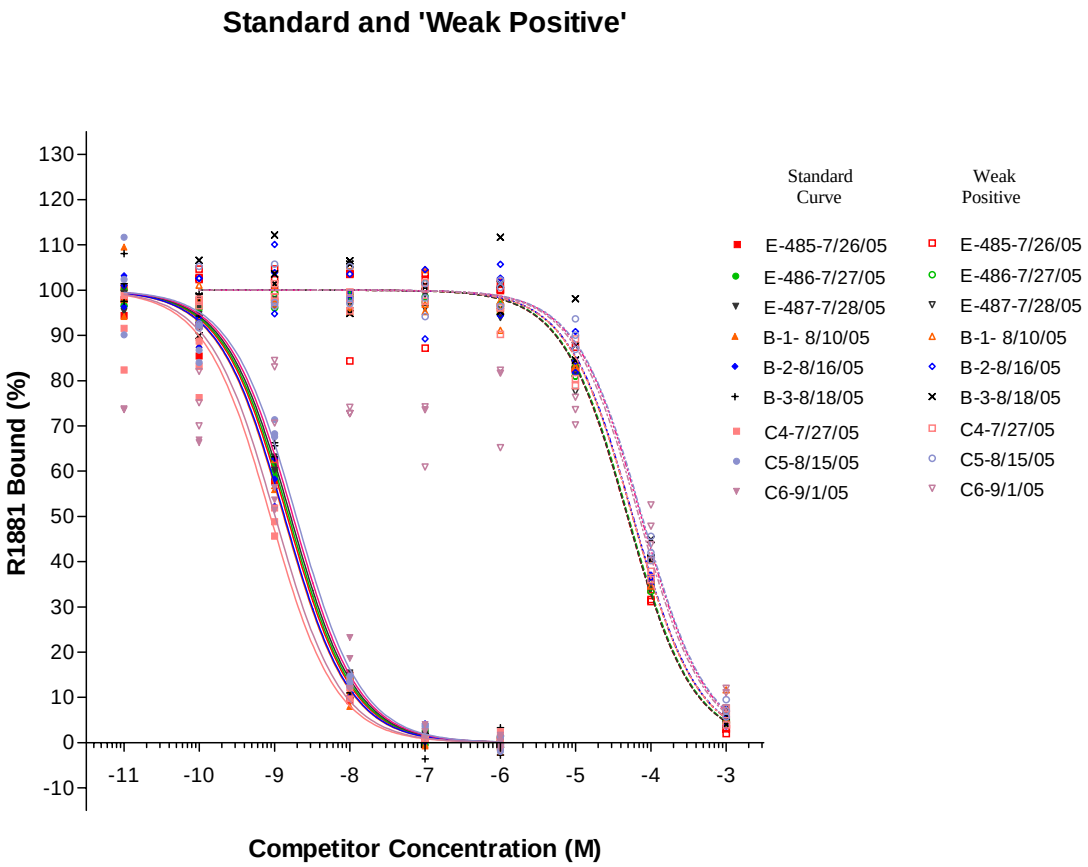

Figure 3. Inter-laboratory variability of one-site competitive curves using all data and model constraints

Appendix A: Saturation binding assay results used to fit the nonlinear one-site binding equation. Note, cells highlighted in yellow were removed by the individual laboratory. The mean total added data is the mean observation for each concentration as per the protocol.

Lab A					
Mean Total Added (Molar)	Specific Bound (Molar)	Mean Total Added (Molar)	Specific Bound (Molar)	Mean Total Added (Molar)	Specific Bound (Molar)
A3-7/18/05	A3-7/18/05	A4-rev-8/16/05	A4-rev-8/16/05	A5-8/23/05	A5-8/23/05
2.34E-10	1.61E-11	2.36E-10	1.22E-11	2.49E-10	1.36E-11
2.34E-10	1.74E-11	2.36E-10	1.45E-11	2.49E-10	1.41E-11
2.34E-10	1.64E-11	2.36E-10	1.54E-11	2.49E-10	1.41E-11
4.78E-10	2.48E-11	4.52E-10	2.26E-11	4.83E-10	2.30E-11
4.78E-10	2.65E-11	4.52E-10	2.56E-11	4.83E-10	1.90E-11
4.78E-10	2.65E-11	4.52E-10	2.37E-11	4.83E-10	2.05E-11
6.81E-10	3.18E-11	6.47E-10	3.09E-11	6.85E-10	2.73E-11
6.81E-10	3.21E-11	6.47E-10	3.07E-11	6.85E-10	3.10E-11
6.81E-10	3.24E-11	6.47E-10	3.06E-11	6.85E-10	3.15E-11
9.73E-10	4.18E-11	9.45E-10	3.21E-11	9.65E-10	3.68E-11
9.73E-10	3.74E-11	9.45E-10	3.47E-11	9.65E-10	3.91E-11
9.73E-10	3.62E-11	9.45E-10	4.08E-11	9.65E-10	3.32E-11
1.40E-09	4.61E-11	1.41E-09	4.49E-11	1.48E-09	4.87E-11
1.40E-09	4.67E-11	1.41E-09	4.30E-11	1.48E-09	4.18E-11
1.40E-09	4.30E-11	1.41E-09	4.56E-11	1.48E-09	5.64E-11
2.49E-09	6.62E-11	2.44E-09	6.76E-11	2.42E-09	7.54E-11
2.49E-09	7.26E-11	2.44E-09	8.32E-11	2.42E-09	8.77E-11
2.49E-09	7.70E-11	2.44E-09	7.89E-11	2.42E-09	7.82E-11
4.58E-09	9.39E-11	4.94E-09	9.24E-11	4.91E-09	1.12E-10
4.58E-09	8.22E-11	4.94E-09	8.13E-11	4.91E-09	8.24E-11
4.58E-09	1.13E-10	4.94E-09	9.78E-11	4.91E-09	1.10E-10
9.40E-09	1.16E-10	9.74E-09	1.04E-10	9.48E-09	1.13E-10
9.40E-09	1.17E-10	9.74E-09		9.48E-09	
9.40E-09	1.04E-10	9.74E-09		9.48E-09	
Within Run Coefficient of Variation					
Mean Total Added (Molar)	CV	Mean Total Added (Molar)	CV	Mean Total Added (Molar)	CV
A3-7/18/05	A3-7/18/05	A4-rev-8/16/05	A4-rev-8/16/05	A5-8/23/05	A5-8/23/05
2.34E-10	3.95%	2.36E-10	11.88%	2.49E-10	2.30%
4.78E-10	3.90%	4.52E-10	6.45%	4.83E-10	9.92%
6.81E-10	0.90%	6.47E-10	0.38%	6.85E-10	7.62%
9.73E-10	7.61%	9.45E-10	12.54%	9.65E-10	8.28%
1.40E-09	4.34%	1.41E-09	2.98%	1.48E-09	14.92%
2.49E-09	7.50%	2.44E-09	10.56%	2.42E-09	8.03%
4.58E-09	15.90%	4.94E-09	9.33%	4.91E-09	16.18%
9.40E-09	6.50%	9.74E-09		9.48E-09	

Appendix A continued

Lab B					
Mean Total Added (Molar)	Specific Bound (Molar)	Mean Total Added (Molar)	Specific Bound (Molar)	Mean Total Added (Molar)	Specific Bound (Molar)
B1-7/5/05	B1-7/5/05	B2-7/7/05	B2-7/7/05	B3-7/11/05	B3-7/11/05
2.20E-10	2.03E-11	2.15E-10	2.40E-11	2.19E-10	2.98E-11
2.20E-10	2.45E-11	2.15E-10	1.98E-11	2.19E-10	2.71E-11
2.20E-10	2.80E-11	2.15E-10	2.16E-11	2.19E-10	3.02E-11
4.39E-10	4.26E-11	4.43E-10	3.54E-11	4.49E-10	3.98E-11
4.39E-10	4.70E-11	4.43E-10	3.86E-11	4.49E-10	4.51E-11
4.39E-10	4.30E-11	4.43E-10	3.92E-11	4.49E-10	3.95E-11
6.36E-10	5.15E-11	6.24E-10	5.03E-11	6.19E-10	4.81E-11
6.36E-10	5.23E-11	6.24E-10	4.41E-11	6.19E-10	5.68E-11
6.36E-10	5.07E-11	6.24E-10	4.75E-11	6.19E-10	4.94E-11
8.64E-10	6.60E-11	8.71E-10	5.91E-11	8.69E-10	6.28E-11
8.64E-10	6.18E-11	8.71E-10	6.01E-11	8.69E-10	6.04E-11
8.64E-10	6.01E-11	8.71E-10	5.99E-11	8.69E-10	5.17E-11
1.33E-09	6.89E-11	1.34E-09	6.77E-11	1.35E-09	6.23E-11
1.33E-09	6.88E-11	1.34E-09	6.97E-11	1.35E-09	7.51E-11
1.33E-09	6.85E-11	1.34E-09	7.10E-11	1.35E-09	6.90E-11
2.37E-09	8.98E-11	2.35E-09	7.77E-11	2.27E-09	9.14E-11
2.37E-09	8.53E-11	2.35E-09	7.72E-11	2.27E-09	8.38E-11
2.37E-09	7.68E-11	2.35E-09	7.44E-11	2.27E-09	8.45E-11
4.00E-09	7.95E-11	4.72E-09	8.92E-11	4.57E-09	1.20E-10
4.00E-09	8.51E-11	4.72E-09	8.84E-11	4.57E-09	9.29E-11
4.00E-09	9.27E-11	4.72E-09	7.52E-11	4.57E-09	9.30E-11
8.96E-09	8.84E-11	9.35E-09	7.39E-11	9.11E-09	1.06E-10
8.96E-09	1.17E-10	9.35E-09	1.05E-10	9.11E-09	8.12E-11
8.96E-09	9.80E-11	9.35E-09	7.95E-11	9.11E-09	1.25E-10
Within Run Coefficient of Variation					
Mean Total Added (Molar)	CV	Mean Total Added (Molar)	CV	Mean Total Added (Molar)	CV
B1-7/5/05	B1-7/5/05	B2-7/7/05	B2-7/7/05	B3-7/11/05	B3-7/11/05
2.20E-10	15.97%	2.15E-10	9.69%	2.19E-10	5.77%
4.39E-10	5.53%	4.43E-10	5.54%	4.49E-10	7.65%
6.36E-10	1.51%	6.24E-10	6.57%	6.19E-10	9.19%
8.64E-10	4.81%	8.71E-10	0.84%	8.69E-10	9.99%
1.33E-09	0.28%	1.34E-09	2.39%	1.35E-09	9.34%
2.37E-09	7.91%	2.35E-09	2.34%	2.27E-09	4.85%
4.00E-09	7.75%	4.72E-09	9.32%	4.57E-09	15.37%
8.96E-09	14.49%	9.35E-09	19.18%	9.11E-09	21.00%

Appendix A continued

Lab C					
Mean Total Added (Molar)	Specific Bound (Molar)	Mean Total Added (Molar)	Specific Bound (Molar)	Mean Total Added (Molar)	Specific Bound (Molar)
C1a-7/6/05	C1a-7/6/05	C2-7/12/05	C2-7/12/05	C3-7/19/05	C3-7/19/05
1.95E-10	5.94E-11	2.13E-10	5.48E-11	2.13E-10	5.85E-11
1.95E-10	5.34E-11	2.13E-10	6.17E-11	2.13E-10	5.87E-11
1.95E-10	5.53E-11	2.13E-10	6.20E-11	2.13E-10	6.01E-11
4.13E-10	9.13E-11	4.51E-10	1.08E-10	4.19E-10	9.18E-11
4.13E-10	9.92E-11	4.51E-10	1.04E-10	4.19E-10	7.30E-11
4.13E-10	8.72E-11	4.51E-10	1.08E-10	4.19E-10	7.41E-11
5.77E-10	1.10E-10	6.29E-10	1.34E-10	6.76E-10	1.26E-10
5.77E-10	1.26E-10	6.29E-10	1.31E-10	6.76E-10	1.20E-10
5.77E-10	1.10E-10	6.29E-10	1.37E-10	6.76E-10	1.26E-10
7.32E-10	1.51E-10	7.47E-10	1.77E-10	9.06E-10	1.36E-10
7.32E-10	1.45E-10	7.47E-10	1.85E-10	9.06E-10	1.53E-10
7.32E-10	1.29E-10	7.47E-10	1.63E-10	9.06E-10	1.52E-10
1.14E-09	1.49E-10	1.22E-09	1.94E-10	1.31E-09	1.71E-10
1.14E-09	1.62E-10	1.22E-09	1.89E-10	1.31E-09	1.63E-10
1.14E-09	1.57E-10	1.22E-09	1.89E-10	1.31E-09	1.66E-10
1.97E-09	2.15E-10	2.13E-09	2.50E-10	2.21E-09	2.07E-10
1.97E-09	2.09E-10	2.13E-09	2.48E-10	2.21E-09	2.16E-10
1.97E-09	1.80E-10	2.13E-09	2.26E-10	2.21E-09	2.27E-10
4.15E-09	2.49E-10	4.57E-09	2.73E-10	4.41E-09	2.55E-10
4.15E-09	2.42E-10	4.57E-09	2.72E-10	4.41E-09	2.67E-10
4.15E-09	2.50E-10	4.57E-09	2.81E-10	4.41E-09	2.39E-10
8.33E-09	2.62E-10	9.25E-09	2.82E-10	8.75E-09	2.57E-10
8.33E-09	2.53E-10	9.25E-09	3.01E-10	8.75E-09	2.89E-10
8.33E-09	2.62E-10	9.25E-09	2.92E-10	8.75E-09	2.66E-10
Within Run Coefficient of Variation					
Mean Total Added (Molar)	CV	Mean Total Added (Molar)	CV	Mean Total Added (Molar)	CV
C1a-7/6/05	C1a-7/6/05	C2-7/12/05	C2-7/12/05	C3-7/19/05	C3-7/19/05
1.95E-10	5.40%	2.13E-10	6.86%	2.13E-10	1.51%
4.13E-10	6.60%	4.51E-10	2.16%	4.19E-10	13.24%
5.77E-10	7.99%	6.29E-10	2.15%	6.76E-10	2.99%
7.32E-10	8.02%	7.47E-10	6.27%	9.06E-10	6.41%
1.14E-09	4.14%	1.22E-09	1.58%	1.31E-09	2.39%
1.97E-09	9.40%	2.13E-09	5.36%	2.21E-09	4.70%
4.15E-09	1.75%	4.57E-09	1.77%	4.41E-09	5.52%
8.33E-09	2.10%	9.25E-09	3.18%	8.75E-09	6.09%

Appendix A continued

Lab D					
Mean Total Added (Molar)	Specific Bound (Molar)	Mean Total Added (Molar)	Specific Bound (Molar)	Mean Total Added (Molar)	Specific Bound (Molar)
D1-8/11/05	D1-8/11/05	D2-8/16/05	D2-8/16/05	D3-8/30/05	D3-8/30/05
2.30E-10	5.80E-12	2.30E-10	3.55E-12		
2.30E-10	6.88E-12	2.30E-10	4.93E-12		
2.30E-10	6.17E-12	2.30E-10	5.04E-12		
4.54E-10	9.34E-12	4.78E-10	4.98E-12	4.74E-10	1.46E-11
4.54E-10	9.08E-12	4.78E-10	7.64E-12	4.74E-10	1.56E-11
4.54E-10	9.42E-12	4.78E-10	6.31E-12	4.74E-10	1.42E-11
6.35E-10	8.37E-12	6.64E-10	9.42E-12		
6.35E-10	9.77E-12	6.64E-10	7.81E-12		
6.35E-10	1.34E-11	6.64E-10	8.47E-12		
8.97E-10	1.02E-11	9.68E-10	1.40E-11	9.39E-10	1.78E-11
8.97E-10	9.52E-12	9.68E-10	9.74E-12	9.39E-10	1.79E-11
8.97E-10	8.76E-12	9.68E-10	1.54E-11	9.39E-10	1.89E-11
1.36E-09	9.45E-12	1.37E-09	8.84E-12		
1.36E-09	9.15E-12	1.37E-09	8.03E-12		
1.36E-09	9.96E-12	1.37E-09	8.76E-12		
2.33E-09	2.05E-12	2.23E-09	1.42E-11		
2.33E-09	1.19E-11	2.23E-09	1.71E-11		
2.33E-09	5.22E-12	2.23E-09	1.46E-11		
4.64E-09	2.52E-11	4.59E-09	1.23E-11	4.48E-09	2.91E-11
4.64E-09	3.66E-11	4.59E-09	1.49E-11	4.48E-09	2.98E-11
4.64E-09	1.92E-11	4.59E-09	1.20E-11	4.48E-09	2.45E-11
9.19E-09	2.70E-11	9.14E-09	1.18E-10	8.97E-09	3.57E-11
9.19E-09	2.43E-10	9.14E-09	5.18E-11	8.97E-09	1.98E-11
9.19E-09	1.20E-10	9.14E-09	1.41E-10	8.97E-09	1.85E-11
Within Run Coefficient of Variation					
Mean Total Added (Molar)	CV	Mean Total Added (Molar)	CV	Mean Total Added (Molar)	CV
D1-8/11/05	D1-8/11/05	D2-8/16/05	D2-8/16/05	D3-8/30/05	D3-8/30/05
2.30E-10	8.72%	2.30E-10	18.45%		
4.54E-10	1.90%	4.78E-10	21.08%	4.74E-10	5.02%
6.35E-10	24.60%	6.64E-10	9.45%		
8.97E-10	7.79%	9.68E-10	22.61%	9.39E-10	3.34%
1.36E-09	4.29%	1.37E-09	5.23%		
2.33E-09	78.53%	2.23E-09	10.40%		
4.64E-09	32.64%	4.59E-09	12.16%	4.48E-09	10.38%
9.19E-09	83.56%	9.14E-09	44.70%	8.97E-09	38.79%

Appendix A continued

Lab E					
Mean Total Added (Molar) E480-7/13/05	Specific Bound (Molar) E480-7/13/05	Mean Total Added (Molar) E484-7/25/05	Specific Bound (Molar) E484-7/25/05	Mean Total Added (Molar) E488-8/1/05	Specific Bound (Molar) E488-8/1/05
2.12E-10	5.31E-11	2.16E-10	4.99E-11	2.10E-10	5.89E-11
2.12E-10	5.15E-11	2.16E-10	4.83E-11	2.10E-10	5.49E-11
2.12E-10	5.39E-11	2.16E-10	5.51E-11	2.10E-10	5.43E-11
4.30E-10	9.97E-11	4.38E-10	1.08E-10	4.31E-10	9.98E-11
4.30E-10	9.62E-11	4.38E-10	9.03E-11	4.31E-10	9.92E-11
4.30E-10	9.58E-11	4.38E-10	1.03E-10	4.31E-10	9.84E-11
6.79E-10	1.07E-10	5.92E-10	9.58E-11	5.89E-10	1.23E-10
6.79E-10	1.08E-10	5.92E-10	9.01E-11	5.89E-10	1.19E-10
6.79E-10	1.14E-10	5.92E-10	8.45E-11	5.89E-10	1.24E-10
8.33E-10	1.47E-10	8.38E-10		8.34E-10	1.53E-10
8.33E-10	1.36E-10	8.38E-10	1.12E-10	8.34E-10	1.50E-10
8.33E-10	1.46E-10	8.38E-10	1.21E-10	8.34E-10	1.51E-10
1.26E-09	1.63E-10	1.28E-09	1.42E-10	1.25E-09	1.84E-10
1.26E-09	1.69E-10	1.28E-09	1.46E-10	1.25E-09	1.81E-10
1.26E-09	1.67E-10	1.28E-09	1.49E-10	1.25E-09	1.84E-10
2.10E-09	2.12E-10	2.28E-09	2.12E-10	2.13E-09	2.19E-10
2.10E-09	2.09E-10	2.28E-09		2.13E-09	2.17E-10
2.10E-09	2.02E-10	2.28E-09	2.24E-10	2.13E-09	2.20E-10
4.29E-09	2.48E-10	4.57E-09	2.54E-10	4.24E-09	2.55E-10
4.29E-09	2.53E-10	4.57E-09	2.64E-10	4.24E-09	2.54E-10
4.29E-09		4.57E-09	1.97E-10	4.24E-09	2.52E-10
8.58E-09		8.91E-09	1.87E-10	8.10E-09	2.72E-10
8.58E-09		8.91E-09	1.93E-10	8.10E-09	2.79E-10
8.58E-09	2.31E-10	8.91E-09	2.63E-10	8.10E-09	2.71E-10
Within Run Coefficient of Variation					
Mean Total Added (Molar) E480-7/13/05	CV E480-7/13/05	Mean Total Added (Molar) E484-7/25/05	CV E484-7/25/05	Mean Total Added (Molar) E488-8/1/05	CV E488-8/1/05
2.12E-10	2.37%	2.16E-10	6.94%	2.10E-10	4.45%
4.30E-10	2.21%	4.38E-10	9.14%	4.31E-10	0.72%
6.79E-10	3.57%	5.92E-10	6.26%	5.89E-10	2.14%
8.33E-10	4.10%	8.38E-10	5.22%	8.34E-10	1.22%
1.26E-09	1.73%	1.28E-09	2.30%	1.25E-09	0.91%
2.10E-09	2.34%	2.28E-09	3.88%	2.13E-09	0.54%
4.29E-09	1.22%	4.57E-09	15.16%	4.24E-09	0.68%
8.58E-09		8.91E-09	19.83%	8.10E-09	1.73%

Appendix B: Competitive binding assay results used to fit the nonlinear one-site binding equation. Note, cells highlighted in yellow were removed by the individual laboratory; cells highlighted in blue were identified as outliers.

Standard Assay									
	Lab B								
	B-1- 8/10/05			B-2-8/16/05			B-3-8/18/05		
Log Final Concentration	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
-6	1.26	1.06	0.30	-1.30	1.57	-1.08	-2.75	-0.29	1.52
-6	-2.11	0.38	-0.89	0.50	0.93	-0.62	3.30	-0.74	-1.04
-7	1.94	-0.42	-0.70	0.14	4.23	0.80	-3.58	1.83	2.09
-8	11.4	8.0	11.0	13.3	10.2	10.2	9.9	14.8	11.0
-9	62.5	55.9	56.8	52.3	58.3	62.8	66.3	65.6	63.2
-10	89.5	87.9	92.3	87.4	98.2	94.0	97.7	94.3	99.1
-11	110	98.1	94.2	96.3	103	101	101	97.5	108
Weak Positive Assay									
	Lab B								
	B-1- 8/10/05			B-2-8/16/05			B-3-8/18/05		
Log_Final Concentration	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
-3	6.09	5.35	11.7	5.88	7.66	3.69	4.05	5.82	7.49
-4	34.6	35.8	40.6	36.6	37.0	35.8	40.8	44.9	40.4
-5	84.7	83.3	82.9	90.8	84.3	81.8	84.5	87.8	98.1
-6	95.5	91.1	97.6	103	106	94.4	101	95.3	112
-7	96.9	105	95.3	105	100	89.2	101	101	101
-8	96.1	105	95.2	104	98.1	99.5	106	94.8	106
-9	101	97.5	97.0	94.8	110	104	101	112	104
-10	93.1	101	98.0	103	92.9	103	107	90.0	98.7

Appendix B continued

Standard Assay									
	Lab C								
	C4-7/27/05			C5-8/15/05			C6-9/1/05		
Log Final Concentration	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
-6	-0.604	-0.560	2.42	1.50	-1.42	0.982	1.15	1.65	-0.654
-6	-0.375	-0.819	-0.068	-1.94	1.13	-0.258	-1.49	0.229	-0.881
-7	1.14	0.863	0.808	3.72	4.03	3.46	3.99	1.07	2.42
-8	9.67	12.1	14.0	12.5	13.4	14.8	23.2	18.6	11.9
-9	45.7	51.9	48.8	68.3	67.6	71.4	56.4	51.4	53.7
-10	76.3	83.2	88.8	84.0	92.2	86.7	66.3	66.9	34.8
-11	91.5	82.4	98.8	112	102	90.1	73.6	73.8	73.6
Weak Positive Assay									
	Lab C								
	C4-7/27/05			C4-7/27/05			C4-7/27/05		
Log_Final Concentration	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
-3	3.92	6.19	7.66	9.51	7.12	5.72	11.0	7.68	12.04
-4	40.3	37.8	36.1	40.3	45.6	42.0	47.8	52.5	43.8
-5	89.3	78.8	80.5	86.9	90.1	93.7	70.2	76.3	73.6
-6	96.2	90.2	96.1	96.5	102	101	65.2	82.3	81.6
-7	102	97.6	100	98.3	101	94.2	74.2	73.5	60.9
-8	98.0	95.5	99.6	97.0	105	98.3	74.1	72.7	72.7
-9	98.6	101	97.6	106	96.5	97.3	84.5	70.6	83.0
-10	98.0	97.6	96.6	105	91.7	92.5	75.1	70.0	82.1

Appendix B continued

Standard Assay									
	Lab E								
	E-485-7/26/05			E-486-7/27/05			E-487-7/28/05		
Log Final Concentration	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
-6	0.654	-1.04	-0.423	-0.655	0.411	-0.630	-0.836		-0.196
-6	0.555	0.613	-0.355	-0.317	1.23	-0.040	0.365	0.296	0.371
-7	0.872	0.759	1.41	2.66	1.76	-0.473	1.13	1.78	2.42
-8	15.0	14.0	9.29	13.8	14.2	14.1	13.1	14.9	15.4
-9	61.8	57.7	62.3	61.8	58.9	60.3	63.2	60.2	62.3
-10	85.5	92.1	92.5	94.3	93.4	95.8	94.6	96.1	91.7
-11	94.4	101	97.9	96.6	100	96.5	100	95.3	99.3
Weak Positive Assay									
	Lab E								
	E-485-7/26/05			E-485-7/26/05			E-485-7/26/05		
Log_Final Concentration	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
-3	2.03	3.97	3.07	4.42	4.85	5.21	5.62	6.15	4.71
-4	31.6	31.2	33.8	35.8	35.2	33.4	34.1	34.6	35.2
-5	87.4	82.8	82.4	81.0	81.0	83.0	77.6	82.2	82.3
-6	100	100	102	96.9	96.6	94.8	93.9	94.7	96.3
-7	102	104	87	97.3	101	98.6	99.1	99.8	96.0
-8	84.4	104	103	99.0	99.0	98.7	97.0	95.5	97.8
-9	105	100	102	99.3	96.1	97.8	96.7	96.8	97.8
-10	103	105	102	96.2	97.8	96.9	96.8	96.5	97.5